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THE MEASUREMENT OF DOG GASTRIC MUCOSAL BLOOD FLOW BY RADIOACTIVE ANILINE CLEARANCE COMPARED WITH AMIDOPYRINE CLEARANCE

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SUMMARY

1. Methods for the estimation of radioactive aniline in body fluids are described. The recovery of aniline added to blood, plasma and gastric juice was over 90% of the recovery from saline.

2. In the doses used aniline caused methaemoglobinaemia of 5–11% of total haemoglobin. No other effect was detected. Gastric secretion was also unaffected.

3. Aniline clearance increased in parallel with acid secretion from Heidenhain pouches in conscious dogs and in anaesthetized dogs. In conscious dogs the ratio of aniline clearance to acid secretion was significantly higher for histamine stimulation than for pentagastrin stimulation.

4. Aniline and amidopyrine clearances were compared simultaneously in the same dogs. Aniline clearance was about 80% of amidopyrine clearance.

5. The proportion of aniline bound to plasma proteins was measured by two methods and found to be 25%. When aniline clearance was corrected for plasma binding, aniline and amidopyrine clearances were equal.

INTRODUCTION

Shore, Brodie & Hogben (1957) demonstrated that amidopyrine, aniline and other weakly basic drugs, with a pK_a = 5–10, were concentrated in acid gastric juice after intravenous administration. They explained these findings by a pH partition hypothesis; at blood pH these bases are lipoid soluble and diffuse freely through cell membranes including the gastric mucosa, but in acid gastric juice they gain a proton and become cations which are not free to diffuse back into the blood. At equilibrium the theoretical concentration in gastric juice of a base with pK_a = 5 is 10^4

times its concentration in plasma but the ratio attained in the dog is 40. It is postulated that equilibrium is not reached because all the base is removed from the gastric mucosal blood in one passage. Hence the clearance of such substances can be used to measure gastric mucosal blood flow.

The use of amidopyrine clearance for the measurement of gastric mucosal blood flow was introduced by Jacobson, Linford & Grossman (1966) who performed an impressive series of experiments validating the technique. Gastric mucosal clearances were discussed in detail by Jacobson (1968) who concluded that amidopyrine was the best available marker substance. There has since been increased interest in gastric mucosal blood flow both in the conscious (Jacobson, Eisenberg & Swan, 1966; Jacobson, Swan & Grossman, 1967; Jacobson & Chang, 1969; Jacobson, 1970; Jacobson & Price, 1969; Cowley & Code, 1970) and in the anaesthetized animal (Moody, 1968; Harper, Reed & Smy, 1968; Reed & Sanders, 1971*a*, *b*). Most workers agree that amidopyrine clearance is a valid measurement of gastric mucosal blood flow under all conditions in which acid is actively secreted. There is less unanimity about the significance of amidopyrine clearance when acid secretion is prevented by thiocyanate (Moody, 1968, 1972) or other drugs.

These problems were discussed by Jacobson (1968) who also pointed out that the amidopyrine clearance method had the disadvantages of a laborious procedure and occasional depression of haematopoietic activity of bone marrow.

The availability of [^{14}C]aniline enabled us to use it instead of amidopyrine as a marker substance to measure gastric mucosal blood flow. The advantages of using radioactive aniline are that it is much simpler to measure and that it does not depress bone marrow function. A disadvantage is that aniline metabolites are capable of producing methaemoglobin. Although in the doses used in our experiments the percentage of haemoglobin oxidized to methaemoglobin was low, this property of aniline prohibits its use in man.

If aniline clearance is to be accepted as a valid and easier alternative to amidopyrine clearance it is necessary to compare the use of the two methods. Some aspects of such a comparison have previously been considered (Curwain & Holton, 1971; Curwain, 1972*a*).

METHODS

Preparation and doses of [^{14}C]aniline

A stock solution containing 35 mg/ml. of carrier aniline (aniline sulphate, B.D.H. Laboratory Reagent Grade) and [^{14}C]aniline 2·0 μCi/ml. (Radiochemical Centre, C.F.A. 71) in sterile saline was prepared. Doses were expressed in mg of carrier aniline per kg body weight.

Aniline loading dose: 10 mg/kg

Aniline maintenance infusion: 10–15 mg kg^{-1} hr^{-1}

The stock solution contained suitable proportions of radioactive and non-radioactive aniline to give satisfactory levels of ^{14}C for counting in the plasma and gastric juice.

Estimation of [^{14}C]aniline in plasma and gastric juice

Heparinized blood was centrifuged at 3000 rev/min for 10 min and the plasma removed and mixed (see Results). 1·0 ml. plasma was added to 0·5 ml. M-NaOH and 10 ml. diethyl ether and shaken for 5 min. 7·0 ml. of the ether phase were removed and added to a bottle containing 10 ml. of scintillator. This was counted for ^{14}C radioactivity in a Packard Tri Carb Liquid Scintillation Spectrometer.

1·0 ml. gastric juice, filtered to remove mucus if necessary, was added to 0·5 ml. M-NaOH and 10 ml. diethyl ether and extracted as above. When the volume of secretion was very small, the juice was diluted with saline and a 1 ml. aliquot extracted.

Scintillator:

Dimethyl POPOP (1,4-bis-[2-(4-methyl-5-phenyloxazolyl benzene)]) 52·5 mg;
PPO (2,5-diphenyloxazole) 4·2 g;
Methanol (I.C.I. Analytical Reagent) 300 ml.;
Toluene (Analar Reagent) 700 ml.

Estimation of amidopyrine in plasma and gastric juice

The method of Brodie & Axelrod (1950) was used to measure amidopyrine in plasma and gastric juice. The recovery of amidopyrine from gastric juice was 98% ± 2·5% (S.E., $n = 6$) and from plasma 95% ± 2·0% (S.E., $n = 45$).

Aniline is extracted with amidopyrine from plasma and gastric juice by alkaline dichloroethane and in the experiments in which both markers were used aniline contributed about 8% to the absorption at 260 nm. Since R (defined below) is similar for the two substances, the readings for plasma and gastric juice amidopyrine were both increased by the same proportion and hence R for amidopyrine was unaffected by the presence of aniline. R is also unaffected by 4-amino antipyrine, the main metabolite of amidopyrine, the free form of which is also extracted with the parent compound.

Calculation of clearance

A graph of [^{14}C] aniline concentration in the plasma against time was constructed. The mean plasma aniline concentration during a period of gastric juice collection was estimated from the graph. Aniline concentrations were expressed as net counts per minute of radioactivity.

Aniline clearance and amidopyrine clearance were calculated from the following formula:

$$\text{Clearance} = \frac{GV}{PT},$$

where

G is concentration in gastric juice,

V is the volume of juice secreted or, when secretion is small, the total volume after dilution,

P is the mean plasma concentration during the collection period,

T is the time in minutes over which the juice was collected.

Two ratios of clearance to secretion have been calculated:

(i) $R = G/P$, the ratio of aniline or amidopyrine concentration in the gastric juice to that in the plasma; and

(ii) μequiv/ml.—This relates gastric acid output to clearance. It is calculated from the rate of acid secretion, measured in μequiv H^+ min^{-1} and the clearance measured in ml./min.

The use of tritiated [^3H]aniline. In some of the later experiments [^{3}H]aniline replaced the ^{14}C compound. This was found to be less expensive and its use was adopted after experiments with both isotopes had shown that identical results were obtained with each. The extraction procedure remained unaltered but more radioactivity was required since tritium is counted with a lower efficiency than ^{14}C. The stock solution (see above) was made up containing 12 μCi ^{3}H/ml.

Dogs

Healthy mongrel bitches weighing 6·8–18 kg were used. Separated (Heidenhain) gastric pouches were constructed and provided with Gregory cannulae. In some of the dogs arterial and venous catheters of polyethylene or silicone rubber were inserted into branches of the femoral artery and vein and passed into the aorta and inferior vena cava. The other ends of the catheters were channelled subcutaneously and brought out through the skin in the interscapular region. The catheters were fitted with needles and stopcocks and were filled with heparin which was replaced about once a week. The operation was carried out under general anaesthesia with full aseptic precautions. Several weeks were allowed for recovery from operation before the animals were used for experiments.

Food was removed from the dogs 20 hr before each experiment. Water was available. During the experiment the dogs stood in slings. If necessary, sterile intravenous catheters were inserted into superficial limb veins.

The indwelling arterial catheter or one of the I.V. catheters was used for withdrawal of blood samples. Aniline and drugs were infused in saline through another I.V. catheter using a Watson Marlow peristaltic pump calibrated on each occasion to deliver 1 ml./min.

Studies in anaesthetized dogs. Anaesthesia was induced with I.V. pentobarbitone (30 mg/kg) and maintained with further doses as required. After tracheal intubation bilateral splanchnectomy was performed, the abdomen opened and separated gastric pouches constructed from the greater curvature. A stainless-steel cannula was secured into the pouch and externalized through a stab wound in the body wall. The spleen was removed and a polyethylene catheter inserted into the splenic vein so that its tip lay at the junction with the vein draining the greater curvature of the pouch. Gentle suction permitted gastric venous blood to be sampled. The abdomen was closed and aniline and amidopyrine infused together through a catheter in the femoral vein. Acid secretion was induced by histamine acid phosphate (4 μg kg^{-1} min^{-1}) added to the infusion. Arterial blood was sampled from a catheter in the femoral artery.

Measurement of gastric acid secretion

The secretion from gastric pouches was allowed to drain under gravity into a collection vessel. Secretion was collected over 5–20 min, the volume measured, and an aliquot titrated against 0·1 M-NaOH with phenolphthalein as the indicator. Acid secretion was calculated in μM-H^+/min.

Excretion of ^{14}C

Each animal received about 50 μCi ^{14}C during an experiment. After the experiment the dogs were kept in a metabolism cage and the urine was monitored. Daily urinary excretion of ^{14}C was estimated as a percentage of the total dose given during the experiment. This estimate also included the ^{14}C in the pouch secretion. About 50%

of the total ^{14}C was recovered in the urine during the first 24 hr and about 5% during the subsequent 4 days. The remaining radioactivity was presumably lost in faeces and urine passed during exercise.

Test meal

The standard test meal (Öbrink, 1954) was prepared from ox liver which was cooked, minced and frozen. On the day of the experiment the required amount (usually 30 g/kg) was thawed and mixed with 20% (w/w) calcium phosphate. The meal was moistened and about 10 g sodium chloride mixed with it. The dogs ate the meal in a few min.

Methaemoglobin estimations

The methods of Evelyn & Malloy (1938) and Dacie & Lewis (1968) were modified as follows.

Solutions

A 6 vol. 0·1 M phosphate buffer pH 6·8 mixed with 4 vol. 1% (v/v) Triton × 100.
B 10% (w/v) sodium cyanide (freshly prepared).
C 20% (w/v) potassium ferricyanide.
D Saturated ferrous sulphate.

Method

Five drops of blood were haemolysed in 10 ml. solution *A*. The optical density was determined at 635 nm (D_1) against solution *A*. The methaemoglobin was then converted to cyanmethaemoglobin by adding 1 drop solution *B*. The optical density was again determined at 635 nm (D_2). Then the total haemoglobin was converted to cyanmethaemoglobin by adding 1 drop solution *C*. The optical density was determined at 540 nm (D_3) against solution *A* containing one drop each of *B* and *C*. The percentage methaemoglobin is then calculated as $0{\cdot}86\ (D_1 - D_2)/D_3$. Solutions containing cyanide were discarded into solution *D*. This method is hazardous. Ingestion of 0·3 ml. of solution *B* would be expected to be toxic. Inadvertent inhalation of its vapour during determinations often causes headache.

Measurement of plasma binding by equilibrium dialysis

Blood was taken with sterile precautions from the dogs, heparinized and centrifuged. To 2 ml. plasma was added 0·1 ml. of a solution containing 3·5 mg aniline/ml. and 0·2 μCi [^{14}C] aniline/ml. The mixture was then placed inside a sterile Visking membrane and dialysed against 38 ml. sterile saline. Dialysis was carried out at −25 mm Hg. This pressure approximated to the osmotic pressure of the plasma proteins and ensured that net movement of water across the membrane was minimal. It was assumed that the free aniline was in dynamic equilibrium with the bound aniline and that the free aniline could diffuse across the membrane. The amount of aniline used was adjusted so that the final concentration inside the membrane was within the range of plasma concentration found during clearance experiments. Dialysis was carried out overnight at 4° C and then for 8 hr at room temperature. Five 1·0 ml. samples were taken for counting from outside the membrane and three 0·5 ml. samples from the plasma inside. A control experiment was carried out with saline instead of plasma inside the membrane. The percentage of aniline bound to plasma protein was calculated from the following formula

$$\% \text{ bound} = \frac{P - F}{P} \times 100,$$

where P is concentration of aniline inside the membrane and F is concentration of aniline outside.

Measurement of plasma binding by ultrafiltration

A modification of the method described by Toribara, Terepka & Dewey (1957) was used. Visking dialysis membrane was boiled for an hour in distilled water. A knot was tied in one end and 7 ml. fresh canine plasma at 37° C and 0·1 ml. of a [^{14}C]aniline solution containing 0·5 mg total aniline/ml. were introduced into the bag which was sealed by tying a knot in the other end. The bag was then placed on a coarse sinter in a centrifuge tube. After equilibration for 30 min at 37° C the bag was centrifuged for 90 min at 2000 rev/min and 37° C in an M.S.E. 6L centrifuge. This manoeuvre produced approximately 1 ml. filtrate from which two 0·5 ml. samples were taken for counting. The contents of the bag, well mixed to ensure homogeneity, were also sampled.

The filtrate was tested for albumin by addition of 3% sulphosalicyclic acid and, if a precipitate showed albumin to be present, the result was discarded. Control experiments were carried out with saline instead of plasma inside the bag. The percentage of aniline bound to plasma protein was calculated as described above.

RESULTS

Recovery of aniline from aqueous solution

Suitable conditions for the extraction of radioactive aniline from aqueous solutions into scintillator were investigated in preliminary experiments. Four solvents, cyclohexane, benzene, diethylether and petroleum ether were shaken with alkaline aqueous aniline as described in Methods. Both benzene and diethylether gave a recovery of more than 98% of radioactivity and diethyl ether was chosen as the solvent for the remainder of the experiments. In a series of eight extractions the mean recovery of radioactive aniline was 98·1% ± 1·8% (S.D.).

Recovery of radioactive aniline added to dogs' gastric juice, blood or plasma in vitro

Blood or gastric juice was taken from pouch dogs which had not received aniline for at least a week. Aniline was added to the heparinized blood or plasma to give a concentration of 7 μg and 9 μCi/ml. and to gastric juice to give 88 μg and 5 μCi/ml. These concentrations are within the usual range of concentrations found in clearance experiments. Samples of gastric juice and plasma were extracted as described in methods and the recovery of aniline was found to be 98% ± 1·0% (S.D., n = 6) from gastric juice and 96% ± 1·2% (S.D., n = 39) from plasma compared with the recovery from saline. The slightly lower recovery from plasma was found not to be due to quenching of the radioactive emissions (Curwain, 1972*b*). When aniline was added to blood before centrifuging and two samples were taken from the supernatant plasma without mixing, the lower sample contained more aniline than the upper sample, probably because the lower sample contained a higher proportion of plasma proteins. When the supernatant

plasma was mixed before sampling the recovery after adding aniline to blood was 92 % ± 1·0 % (S.D., $n = 5$) of the recovery from saline. This is significantly lower than the recovery when aniline was added to plasma. The difference can be explained by assuming that the highest concentration of aniline occurred in the densest part of the plasma which was trapped between the packed cells. Attempts to extract whole blood or packed cells gave very poor recoveries of aniline. The aniline in packed cells was estimated by equilibrating the cells with saline and measuring the aniline in the saline after centrifugation according to the method described by Harper *et al.* (1968). Equilibrium was attained in less than 1·75 min. The recovery was 99 % ± 2·0 % (S.D., $n = 6$) of what would be expected if the concentration of aniline were equal in cells and plasma.

Recovery of radioactive aniline from blood after injection into dogs

If a radioactive substance were completely inert, not metabolized, and not bound to tissues it would suffice to administer a very small weight provided it contained enough radioactivity after dilution in the body. Therefore in preliminary experiments various priming and maintenance doses of aniline, from 2 to 100 % of the doses finally chosen, were used. Reasonable plasma levels were obtained only when the maintenance dose was 10 mg kg^{-1} hr^{-1}. When there was a high rate of secretion from the pouch the amount of aniline lost from the animal in the gastric juice was sufficient to decrease plasma levels below those required for accurate estimation. Therefore in these experiments aniline was infused at 12 or 15 mg kg^{-1} hr^{-1}. Priming doses of 2·5, 5 or 10 mg kg^{-1} were suitable for different dogs. The concentration of aniline in samples of plasma taken during experiments in which [^{14}C]aniline was used were 4–12 μg $ml.^{-1}$ and 300–1000 counts min^{-1} $ml.^{-1}$. The plasma levels were similar to those found by Shore *et al.* (1957) and were about 40 % of the levels that would be expected for amidopyrine given in the same way. Compared with amidopyrine aniline is relatively rapidly metabolized in the dog, mainly to *o*- and *p*-aminophenol (Parke & Williams, 1956). Since these and other possible metabolites of aniline would be radioactive, in our experiments a pH greater than 11 was used in our extraction procedure. At this pH both *o*- and *p*-aminophenols with pK's 9·7 and 10·3 would remain in the aqueous phase and only aniline itself would be extracted. However, in one experiment identical results were obtained from extraction at pH 7 and from extraction at pH > 11.

Toxicity of aniline

Methaemoglobin. Blood taken before giving aniline contained about 2 % methaemoglobin. During the aniline infusion the percentage of methaemo-

globin rose to 6–11 % as shown in Fig. 1. No acute or chronic toxic effects were observed even in dogs which were used repeatedly for aniline experiments for more than a year. The dogs maintained their weights and were very active during exercise periods. Normal haematocrit levels were maintained.

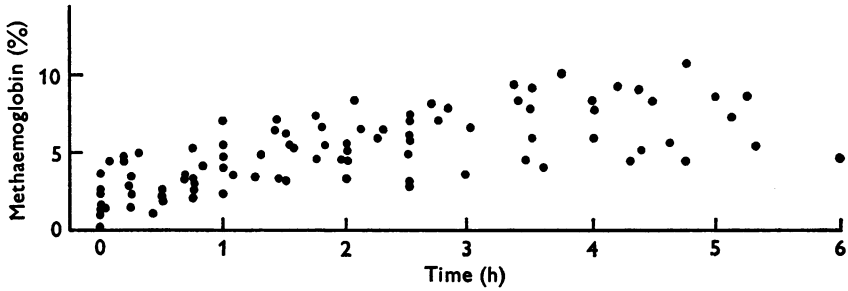

Fig. 1. Methaemoglobin levels expressed as % of total haemoglobin during aniline infusions in ten experiments in five dogs. The mean methaemoglobin omitting the control levels at zero time was 5·5 %. Abscissae: time in hr.

Lack of effect of aniline on gastric secretion. The response to pentagastrin or histamine infusion and standard meals was the same in the presence and absence of aniline (Curwain, 1972*b*).

Aniline clearance during secretion induced by increasing doses of pentagastrin or histamine

In these experiments the dose of secretagogue was doubled every hour until the maximal secretory response was obtained. The results are shown in Fig. 2 in which acid secretion, aniline clearance and the ratio of clearance to secretion have been plotted against time. It can be seen that during pentagastrin infusion aniline clearance followed secretion very closely and the ratio of clearance to the volume of secretion (G/P) and to the rate of acid secretion was constant. During histamine infusion clearance and secretion increased together but at high rates of secretion the ratio of clearance to secretion increased. This difference between the effects of pentagastrin and histamine on the ratio of clearance to secretion is also shown in Fig. 3 in which clearance has been plotted against acid secretion for a large number of observations. The calculated regression coefficients from the points shown in Fig. 3 are 0·34 ml./μmole for histamine and 0·14 ml./μmole for pentagastrin and feeding, corresponding approximately to $G/P = 46$ (histamine) and $G/P = 21$ (pentagastrin). These regression coefficients were significantly different ($t = 13$, $P < 0{\cdot}01$) but there was no difference between the ratios for pentagastrin and feeding (Curwain, 1973).

Comparison of aniline and amidopyrine clearance during simultaneous measurement

In conscious dogs. In a series of seven experiments the secretion and clearances in response to a standard meal were studied. The results of a typical experiment are shown in Fig. 4. During brisk secretion both aniline and amidopyrine clearance were closely related to the acid secretory response but aniline clearance was consistently lower than amidopyrine

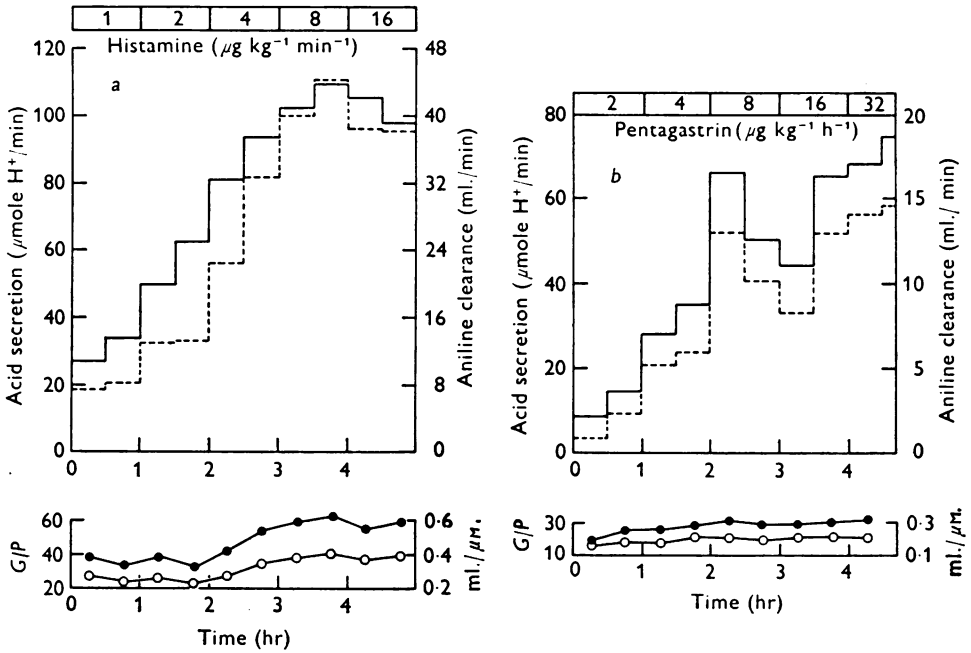

Fig. 2. Aniline clearance (broken line), gastric secretion (continuous line), and the ratios of aniline clearance to the rate of secretory volume G/P (●) and to the rate of acid secretion ml./μmole (○) in Heidenhain pouch dogs during stimulation with I.V. histamine (Fig. 2*a*) or pentagastrin (Fig. 2*b*). The dose of secretagogue was doubled every hour until the maximum response was obtained. Abscissae: time in hr.

clearance. In this experiment an infusion of isoprenaline sulphate (0·25 μg kg^{-1} min^{-1}) was given during secretion. The isoprenaline inhibited secretion and decreased the clearances of both aniline and amidopyrine but increased the ratios of clearance to secretion. Similar results were obtained with isoprenaline in ten experiments. Noradrenaline (1–10 μg/kg) was also given during secretion in six experiments. It decreased secretion and the clearances of both markers. It either decreased or did not affect

the ratios of clearance to secretion. On every occasion aniline and amidopyrine clearances were affected in the same way. These effects of catecholamines have been discussed elsewhere (Curwain & Holton, 1972) but are mentioned here to illustrate that the clearances of aniline and amidopyrine are affected similarly in different conditions.

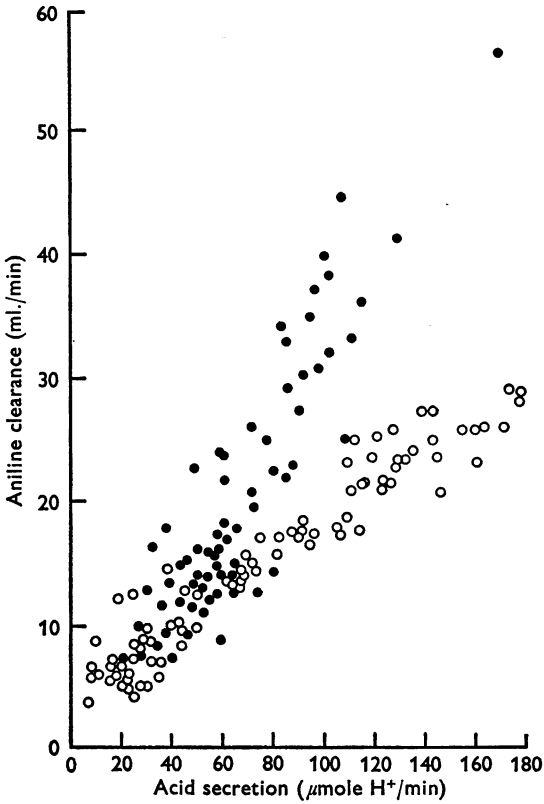

Fig. 3. The ratio of aniline clearance to acid secretion in twenty experiments on seven dogs (179 observations). Ordinate: aniline clearance ml./min; abscissa: rate of acid secretion μmole H^+/min. The calculated regression coefficients 0·34 ml./μmole for histamine (filled circles) and 0·14 ml./μmole for pentagastrin and feeding (open circles) are significantly different ($t = 13$, $P < 0{\cdot}01$).

In the experiment illustrated in Fig. 4 the ratio of aniline clearance to amidopyrine clearance during the period of brisk uninhibited secretion was 83%. Similar ratios were obtained in the other six experiments and are shown for all the experiments in Table 1.

The mean ratio was $74 \pm 19\%$ (S.D., $n = 31$). Analysis of variance showed that there were highly significant differences between dogs

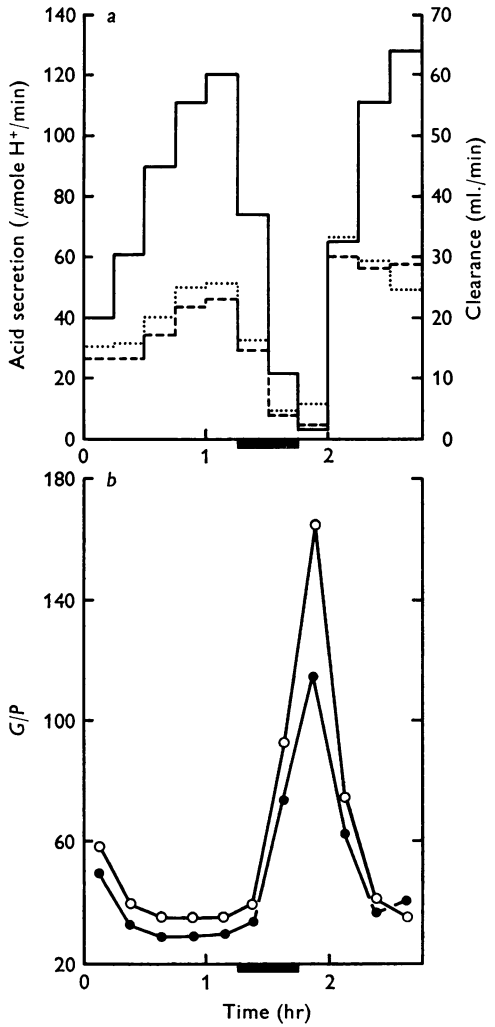

Fig. 4. Histograms of gastric acid secretion (continuous line), aniline clearance (interrupted line) and amidopyrine clearance (dotted line) from a Heidenhain pouch in a conscious dog in response to eating a standard meal given ¾ hr before the beginning of the record. The ratios of aniline clearance (filled circles) and amidopyrine clearance (open circles) to the rate of gastric secretion (G/P) are shown below. During the period indicated isoprenaline sulphate (0·25 μg kg^{-1} min^{-1}) was infused I.V. (black bar). It inhibited acid secretion and decreased the clearances but increased the ratios of clearance to secretion. Abscissae: time in hr.

($F = 7{\cdot}7$, $P < 0{\cdot}001$) and between days in the same dog ($F = 22$, $P < 0{\cdot}001$).

In anaesthetized dogs. In these experiments the gastric venous concen-

TABLE 1. The percentage of *R* (aniline) to *R* (amidopyrine) in Heidenhain pouch dogs after feeding. Aniline and amidopyrine were administered together and the results were obtained by analysis of gastric juice collected over 15 min, analysed for aniline and amidopyrine and compared with the mean plasma concentration of each substance during this time

	Grocer		Peta		Alex		Chris
Dogs	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1
	82·1	86·2	103·6	61·8	64·3	70·0	64·7
	65·8	92·5	100·0	66·7	68·3	64·3	63·9
	65·4	94·1	111·5	68·2	71·8	61·7	59·0
	67·1	106·5	—	68·9	70·7	82·5	—
	—	78·6	—	68·2	—	83·3	—
	—	—	—	—	—	83·3	—
	—	—	—	—	—	63·2	—
Mean ± S.D.	70·1	91·6	105·0	66·8	68·8	72·6	62·5
	8·0	10·3	5·9	2·9	3·3	10·1	3·1

TABLE 2. Gastric venous concentrations of aniline and amidopyrine, expressed as percentages of arterial concentration, in anaesthetized puppies

Expt.	Aniline	Mean	Amidopyrine	Mean
1	36	36·5	28	26·5
	36		27	
	36		26	
	36		26	
	36		26	
	36		26	
	37		26	
	37		27	
	39		27	
2	36	38·0	29	29·0
	36		29	
	38		29	
	42		29	
3	29	29·4	20	20·8
	29		21	
	29		21	
	30		21	
	30		21	
4	45	44·0	44	34·3
	44		37	
	44		32	
	43		24	
Mean	37	37	27	28

trations of aniline and amidopyrine were measured as well as the acid secretion and the clearances of both substances. Secretion was stimulated by histamine (4 μg histamine acid phosphate kg^{-1} min^{-1} I.V.). The results of a typical experiment are illustrated in Fig. 5 which shows that they were very similar to the results in conscious dogs. From the concentrations of aniline and amidopyrine in arterial and gastric venous blood it was

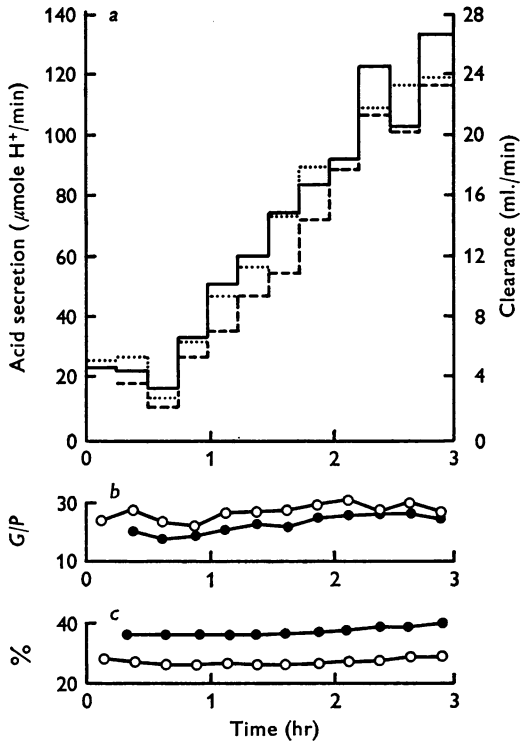

Fig. 5*a*: gastric acid secretion (continuous line), aniline clearance (interrupted line) and amidopyrine clearance (dotted line) in an anaesthetized dog during an infusion of histamine acid phosphate (4 μg kg^{-1} min^{-1}). The splanchnic nerves had been sectioned before laparotomy.

b: the ratios of aniline clearance (filled circles) and amidopyrine clearance (open circles) to the rate of gastric secretion *G*/*P*.

c: gastric venous concentrations of aniline (filled circles) and amidopyrine (open circles) expressed as a percentage of arterial concentrations. Abscissae: time in hr.

possible to calculate the percentage of total gastric blood flow which was cleared of these substances. The results are given in Table 2. The mean value was 67·5% for aniline and 75·3% for amidopyrine. These figures indicate that both aniline and amidopyrine must be removed from the

cells as well as from the plasma in the mucosal circulation. They show further that amidopyrine clearance was 16% higher than aniline clearance in these experiments.

Plasma binding of aniline

The percentage of aniline which was bound to plasma proteins was found to be 26·7% ± 2·4% (S.E.) when measured by equilibrium dialysis (six experiments with plasma from six dogs) and 24·8 ± 2·6% (S.E.) when measured by ultrafiltration (seven experiments with plasma from four dogs). The results of the two different methods are not significantly different and agree with the value of 25% reported by Shore *et al.* (1957).

DISCUSSION

Compared with amidopyrine, radioactive aniline is much more easily measured in body fluids. In our hands the reproducibility of extraction is greater for aniline than for amidopyrine (Curwain, 1972*a*). Therefore if aniline clearance were as high as amidopyrine clearance it would obviously be a better method for the determination of gastric mucosal blood flow. In fact aniline clearance is less than simultaneously determined amidopyrine clearance but this does not preclude its use in clearly defined conditions.

The experiments illustrated in Figs. 2–5 indicate that aniline clearance like amidopyrine clearance increases with the rate of acid secretion. In these experiments the ratio of aniline concentrations in gastric juice and plasma ($R = G/P$) varies from 20 to 60, which is similar to the range found by Jacobson, Linford & Grossman (1966) for amidopyrine. During stimulation by increasing doses of pentagastrin R remained constant at 20–30 until the maximum response was obtained. When increasing doses of histamine were used, however, R became greater than 40 when responses were more than about 60% of maximal. Even with smaller rates of histamine-induced secretion R was higher, 30–40, than for pentagastrin-induced secretion (Curwain, 1973). Jacobson (1968) rejected the possibility that amidopyrine transport was linked with acid secretion on the grounds that R did not increase as acid concentration increased. Our results do not provide contrary evidence. The increase in R that we have observed occurs only at high secretory rates when acid concentration is not related to secretory rate but remains constant as secretory rate is increased. A higher R for histamine-induced secretion than for pentagastrin-induced secretion was observed also by Jacobson & Chang (1969) and Reed & Smy (1971). It can be explained by assuming that histamine has a vasodilator effect over and above the vasodilatation concomitant with secretion. Pentagastrin, however, is not a general vasodilator and the increase in blood flow accompanying pentagastrin-induced secretion is probably secondary to the

metabolic demands of the glands. The fact that R remains constant or increases as secretion increases to a maximum is important evidence that aniline is a suitable marker for measuring changes in gastric mucosal blood flow. It indicates that aniline clearance is not limited by the rate of diffusion from blood to gastric lumen and must therefore be limited by the blood flow. In this respect aniline is superior to radioactive iodide because iodide clearance decreases at high secretory rates (Brown-Grant, Cumming, Haigh & Harries, 1965).

When aniline and amidopyrine clearance were measured simultaneously aniline clearance was about 80% of amidopyrine clearance. Since there is no difficulty in estimating each marker in the presence of the other this result implies that not all the aniline is removed from the blood in a single passage through the mucosal capillaries and hence that in these conditions aniline clearance is not equal to mucosal blood flow.

Since we have no reason to believe that aniline clearance is lower in the presence of amidopyrine than in its absence, it is likely that the 20% aniline which is not removed from the blood is not free to diffuse from plasma to gastric lumen. This proportion is very similar to the proportion of aniline bound to plasma proteins. Therefore it is likely that the protein-bound aniline which is included in the measured plasma aniline does not contribute to the aniline in gastric juice. Mucosal blood flow would therefore be equal to aniline clearance corrected for plasma binding or MBF = $GV/(P-P$ bound) (where P bound is the amount of aniline bound to plasma proteins). When this correction is applied using the measured plasma binding aniline clearance is equal to amidopyrine clearance.

Shore *et al.* (1957) found that both bases were bound to plasma proteins. In their experiments, 25% aniline and 15% amidopyrine were bound. They considered that it was unlikely that plasma binding affected the free diffusion of aniline because in a single experiment on an anaesthetized dog they found that 64% of aniline was removed from blood passing through the gastric circulation and they regarded this as a reasonable proportion of total gastric blood flow for the mucosa. In similar experiments with aniline we obtained a clearance of $67{\cdot}5\% \pm 5{\cdot}4\%$ (S.D., $n = 34$) which agrees with their clearance of 64%. However, the clearance is significantly different ($t = 6{\cdot}1$, $P < 0{\cdot}01$) from that of amidopyrine which was $75{\cdot}3\% \pm 3{\cdot}4\%$ (S.D., $n = 22$) measured simultaneously. The most likely explanation of this difference is the greater plasma binding of aniline.

Does plasma binding affect amidopyrine clearance also? In experiments in which both substances are used together the difference between the clearances can be approximately accounted for by the differences in plasma binding. It is possible that in these conditions aniline may displace amidopyrine from binding sites thus increasing the diffusible

amidopyrine. Our figure of 75% clearance of amidopyrine in the presence of aniline can be compared with the figures obtained by other groups for amidopyrine alone: in anaesthetized dogs 58% (Jacobson, Linford & Grossman, 1966) and 59% (Moody, 1968); in conscious dogs 53% (Jacobson, Eisenberg & Swan, 1966); in anaesthetized cats up to 90% (calculated from Fig. 6 of Harper *et al.* 1968). Therefore our percentage amidopyrine clearance is higher than has been found by other workers for dogs which is compatible with the possibility that in the absence of aniline amidopyrine diffusion is limited by plasma binding and that binding is less in the presence of aniline. There is no information about plasma binding of amidopyrine in cats but it clearly does not affect the very high percentage clearance obtained in this species.

As has been shown above, uncorrected values of aniline clearance are not equal to mucosal blood flow. However, there is evidence that changes in aniline clearance are exactly parallel with changes in amidopyrine clearance. Therefore if we assume that amidopyrine clearance is equal to mucosal blood flow, aniline clearance must equal a proportion of mucosal blood flow which is constant during an experiment. The factor of proportionality may be determined by measuring plasma binding if absolute levels of blood flow are desired. For many experiments it is sufficient to determine changes in mucosal blood flow. For this purpose aniline clearance is superior to amidopyrine clearance because it is simpler. Since the sensitivity of aniline clearance measurements can be easily increased by increasing the proportion of labelled aniline the method can be adapted for small animals (Main & Whittle, 1972).

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